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Exploration of Binding and Toxic Site of Botulinum Neurotoxin.

Annual Report

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Roles of the heavy and light chains of the neurotoxin (NT) in the neuroparalysis were examined in two systems. 1) The heavy chain first binds to the specific sites (receptors) on the nerve terminals. This binding is necessary for the light chain to be fixed at these specific sites. Then the light chain (or combination of the light and heavy chain) induces paralysis through a mechanism very similar to that of the parent dichain NT. 2) The isolated heavy chain forms channels in planar bilayer membranes. These channels have pH and voltage dependent gating properties. Planter nerves - lumbrical of the hind paw of the mouse was introduced as neuromuscular preparation for studying botulinum NT induced paralysis.

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Summary

Roles of the heavy and light chains of the neurotoxin (NT) in the neuroparalysis were examined in two systems. i) The heavy chain first binds to the specific sites (receptors) on the nerve terminals. This binding is necessary for the light chain to be fixed at these specific sites. Then the light chain (or combination of the light and heavy chain) induces paralysis through a mechanism very similar to that of the parent dichain NT. ii) The isolated heavy chain formed channels in planar bilayer membranes. These channels have pH and voltage dependent gating properties. Planter nerves - lumbrical of the hind paw of the mouse was introduced as neuromuscular preparation for studying botulinum NT induced paralysis.

Body of the report (Text)

Research activities under the broad goals of the contract entitled "Exploration of binding and toxic sites of botulinum neurotoxin" were primarily designed to determine the role of the heavy and light chains of the neurotoxin (NT) in the neuroparalysis.

1. The first phase of the studies on the role of the heavy and light chains of the NT were completed. Conclusions drawn are as follows.

   The paralysis of neuromuscular preparations (mouse phrenic-nerve hemidiaphragm) induced by the dichain NT was delayed (antagonized) if the neuromuscular preparations were incubated with the isolated and purified heavy chain prior to or during incubation with the parent dichain NT. Neuromuscular preparations became paralyzed when incubated with the heavy chain, then washed free of nonbound heavy chain, and then further incubated with the light chain. The paralysis did not occur if the neuromuscular preparation was incubated first with the light and then with the heavy chain. These observations make a persuasive case that the heavy chain first binds to the specific sites (receptors) on the nerve terminals. This binding is necessary for the light chain to be fixed at these specific sites. Then the light chain (or combinations of the light and heavy chain) in some way induces paralysis through a mechanism very similar to that of the parent dichain NT. This general model of the structure-function relationship in the mode of action of botulinum NT was developed with types A and B NT, rather than one NT type. Results using the two types are consistent. This is the first direct experimental demonstration of the role of the two subunit chains of the NT.

   A manuscript was written and submitted to a refereed journal for publication as a paper.

   Other laboratories have used brain synaptosome preparation to demonstrate competition for similar sites. It is still unclear from published literature whether the ability of the NT to bind synaptosomes represents the potential toxicity. Also published data does not indicate whether the "receptors" on brain synaptosomes that "bind" botulinum NT are the same as the receptors in NMJ on peripheral nerves.

2. In collaboration with Prof. Alan Finkelstein, we have found that the purified heavy chain (mol. wt. 102,000) derived from type B dichain NT (mol. wt. 152,000) formed channels in planar bilayer membranes. These channels have pH dependent and voltage dependent gating properties similar to channels formed by the heavy chain from diphtheria toxin and the heavy chain from tetanus toxin. The light chain derived from the type B NT and the intact single chain NT under these conditions was devoid of channel forming activity. A manuscript was submitted for publication as a full paper.

3. The work done on the Lumbrical muscle preparations for the study of botulinum neurotoxin (NT) was presented at the Federation of American Societies for Experimental Biology in April 1985 [Fed. Proceed. 44, (#4), 894 (1985)]. A manuscript covering the work was written and submitted to J. Neuroscience methods. Conclusions drawn from the work were presented in the annual report (1 Feb. 83 - 31 Jan. 85).

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